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Temperature acclimation in Tribolium confusum and Musca domestica: rate of acclimation measured at locomotory, metabolic and enzyme levels

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TEMPERATURE ACCLIMATION IN *TRIBOLIUM CONFUSUM* AND *MUSCA DOMESTICA*: RATE OF ACCLIMATION MEASURED AT LOCOMOTORY, METABOLIC AND ENZYME LEVELS

by

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Dean of Graduate College

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INTRODUCTION AND REVIEW

Temperature is an environmental variable which affects all animals. Insects, like other poikilotherms, respond to increases in temperature by increasing the rate of their life processes, and to decreases in temperature by decreasing the rate of such life processes. As the temperature decreases, a temperature is reached at which the insect is inactivated by cold. If the insect is first conditioned at a moderately low temperature, homeostatic compensations may occur which allow the insect to remain active at the low temperature that would otherwise have inactivated it. The temperature at which the insect is inactivated is called the chill-coma temperature. The chill-coma temperature depends on at least two factors: the species of insect and its temperature history.

It is well documented that insect species vary considerably in their resistance to cold. For example, the firebrat, Thermobia domestica, which normally lives in areas around furnaces and steam tunnels, is inactivated by temperatures lower than 10°C (Edwards and Nutting, 1950). Certain midges (Chironomidae) from Baffin Island in Northern Canada are able to complete their life cycles at temperatures below 4.9°C, and adults have emerged at temperatures as low as 0.9°C (Downes, 1962). These species are examples of those insects that function at the extremes of the normal temperature range. Work by Mellanby (1939, 1940), Colhoun (1954, 1960) and Mutchmor and Richards (1961), with insects whose habitat temperatures are well within the normal temperature range, has shown species differences with respect to functional capability at low temperatures. In general, they found that insects which normally encounter a wide range of temperatures have the lowest chill-coma temperatures.

Of even greater relevance to this study is the effect of the previous temperature history on the response of insects to low temperature. Colhoun (1960), Mutchmor and Richards (1961), Thiessen and Mutchmor (1967), and Anderson and Mutchmor (1968) have shown that, if individuals of a species are maintained at two different temperatures, the chill-coma temperature of the insects exposed to the lower temperature may be as much as 5°C lower than that of insects which were maintained at a higher temperature.

Several terms are used to describe the differences between interspecific and intraspecific responses of poikilotherms to temperature. The term adaptation usually refers to responses which have been fixed genetically in the population. Two other terms, acclimatization and acclimation, are also often seen. Acclimatization usually refers to compensations which occur in response to seasonal changes in the environment, whereas acclimation usually refers to compensations which occur in individuals kept under laboratory conditions. Although the two terms differ in meaning they are often used interchangeably. It is possible to detect adaptive differences in chill-coma temperatures between species and, also, acclimative differences in chill-coma temperatures within a species.

It has been a general practice in the study of the effects of temperature adaptation and acclimation to measure the rate of some process as a function of temperature, and then to construct temperature-rate (T-R) curves. Curves for different species or for individuals with different temperature histories are then compared to determine if any adaptive or acclimative relationships exist. Prosser (1961) has placed the results

which may be obtained into five categories. These categories refer, basically, to a translation, which is a movement of the T-R curve along the temperature axis following acclimation; or a rotation of the T-R curve, either in a clockwise or counter-clockwise direction, so that a change in slope results. The other categories are: translation with clockwise rotation; translation with counter-clockwise rotation; and, no adaptation or acclimation.

Two schools of thought have evolved regarding interpretations of T-R curves from poikilotherms. Scholander, et al. (1953) compared the oxygen consumption of a wide range of arctic and tropical insects, spiders and molluscs and concluded that adaptive differences occurred as displacements, i.e., translations, of the T-R curves. Scholander, et al. also recorded that the slopes of the curves of the arctic animals were higher than those of the tropical forms, but they did not consider this to be of adaptive significance. Bullock (1955) in an excellent early review, Prosser (1958), and Precht (1958) report that cold-acclimated poikilotherms usually have rate curves with reduced slopes. Such poikilotherms would be less affected by changing temperatures. In short, cold acclimation involves a reduction in the temperature dependence of metabolic rates.

The differences in response which occur upon adaptation or acclimation raise a basic question. What mechanisms underlie the change in the ability to respond to low temperatures? Several approaches have been used in attempts to answer this question.

In the Insecta, initial studies involved the measurement of oxygen consumed by thermally acclimated insects. The first such definitive work

was done by Dehnel and Segal (1956). They acclimated the American cockroach, Periplaneta americana, to 10, 16, and 26°C. The cockroach acclimated to 10°C consumed more oxygen per gram than the cockroach acclimated to 26°C when measured at the same temperature. This is the most common type of response to temperature acclimation. In general, rate processes of cold-acclimated insects are greater than those of warm-acclimated insects when tested at the same temperature.

Work has also been done to examine the effect of temperature acclimation on certain enzymes of insects and other poikilotherms. Precht, et al. (1955), Bullock (1955), Prosser (1955, 1958) and Mutchmor (1967) have reviewed this field.

Initial enzyme studies with insects explored the effect of temperature acclimation on succinic dehydrogenase, catalase, glycerophosophatase and cocarboxylase (Marzusch 1952, Carlson 1953, Precht 1953). Another enzyme which was studied early and has since been studied extensively is part of the muscle ATPase (adenosine triphosphatase) system. This enzyme system is of great interest because it is considered to be the chief immediate source of energy required for muscle contraction. For this reason, it is thought possible that there may be a relationship between the effects of temperature on ATPase activity and adaptive and acclimative differences in muscular response to temperature.

Many interspecific studies have been done in an attempt to correlate ATPase activity with habitat temperatures. Several of the earlier studies gave inconclusive results. Steinbach (1949) indicated that there was no correlation between the temperature coefficient of ATPase activity and environmental temperature in four species of fish, a sparrow, mouse,

turtle, and frog. Davison and Richards (1954) compared the cockroach, crayfish, and minnow and found the highest ATPase activity in the minnow and the lowest in the cockroach. The highest temperature coefficient was obtained with the cockroach, the lowest with the minnow. Mutchmor and Richards (1961) established a relationship between temperature tolerance and ATPase activity among six species of insects, a crayfish, Cambarus diogenes, and the horseshoe crab, Limulus polyphemus. They found that animals with lower chill-coma temperatures had higher temperature coefficients and lower optimum temperatures for ATPase activity.

There have been few intraspecific examinations of ATPase activity in insects. Mutchmor and Richards (1961) acclimated the mealworm, Tenebrio molitor, to 35°C and 15°C and the cockroach, P. americana to 30, 10, or 15°C. Differences in chill-coma temperatures indicated that acclimation had occurred. They found that the absolute rate of ATPase activity was greater in the cold-acclimated insects but there was little or no effect on the temperature coefficient. Thiessen and Mutchmor (1967) acclimated the housefly, Musca domestica, and the American cockroach, P. americana, and found that at given temperatures the ATPase activity was higher in the cold than in the warm-acclimated insects. It was also shown that the temperature coefficients were lower in the cold-acclimated insects. This is opposite to the relationship found in interspecific comparisons of insects (Mutchmor and Richards, 1961), but similar to the relationship shown for other animals and processes by Bullock (1955), Prosser (1958), and Precht (1958).

Other workers using different muscles in individuals of a species, have also shown the temperature dependence of ATPases. Gilmour and Calaby

(1952, 1953) found a greater activity in flight muscle than in the leg muscle in the locust, Locusta migratoria. The temperature coefficient for flight muscle was also high, and it is thought that this may explain the great temperature dependence of locust flight. A similar study by Kenney and Richards (1955) with the giant water bug, Lethocerus americana, showed that the swimming muscle had a higher temperature coefficient than flight muscle. Flight muscle was inactivated at 10°C, but swimming continued until the temperature reached almost 0°C.

Although there is substantial evidence, based on differences in chill-coma temperatures, oxygen consumption and enzyme activity, that certain insects acclimate to different temperatures, there have been few studies on the rate of acclimation in these animals. Those workers who do report rate of acclimation data are usually only concerned with one aspect of temperature acclimation. The present study is the first attempt to correlate the rate of temperature acclimation at the locomotory, metabolic, and enzyme levels.

Rate of acclimation at the locomotory level has been studied by Bělehrádek (1935), who indicated that differences in chill-coma temperatures occurred after three days at a new temperature. In the blowfly, Calliphora erythrocephala, the tsetse fly, Glossina palpalis, and the bedbug, Cimex lectularis, chill-coma differences could be seen after 20 hours at a new temperature (Mellanby, 1939). Mellanby also reported that, for the Oriental cockroach, Blatta orientalis, two to three days were necessary for acclimation after transfer from 30°C to 15°C. Colhoun (1960) transferred the German cockroach, Blattella germanica, from high to low temperatures and from low to high temperatures. His results are of inter-

est because the rates of acclimation depended on size and range of the temperature change. For example, if the insect was transferred from 25°C to 15°C acclimation was complete within two to three hours. A transfer from 35°C to 25°C or 35°C to 15°C required more than 24 hours for completion. A similar relationship was found in transfers of insects from low to higher temperatures. The transfer of insects from 15°C to 25°C was complete in 16 hours, from 25°C to 35°C in 20 hours, and from 15°C to 35°C in 40 hours.

Edwards (1958) maintained the confused flour beetle, Tribolium confusum, at 18, 30, and 38°C and measured the oxygen consumption of the beetles at a series of temperatures. He examined the T-R curves of beetles from the three maintenance temperatures with respect to differences in the beetles' sex, weight, and water content. As a part of this study, insects from 30°C were transferred to 18°C and to 38°C. He then recorded the oxygen consumption at daily intervals and reported that no acclimation occurred. After 24 hours the rate for beetles transferred to 18°C was the same as that of beetles maintained and tested at 18°C. The transfer of beetles from 30°C to 38°C resulted in an overshoot in oxygen consumption which lasted for 3 days before returning to the baseline rate established for insects maintained and tested at 38°C.

There have been few studies on the rate of acclimation of enzymes in insects. Mutchmor and Richards (1961) maintained the American cockroach, P. americana, at 30°C and 10°C or 15°C for periods of three to ten days, and T. molitor at 35°C and 15°C for a similar length of time before they tested for ATPase activity. Applebaum et al. (1964) transferred T. molitor from 23°C to 13°C and then studied the activity of the midgut pro-

teases and amylases. They found a lag period of about four days after transfer, during which the enzyme activity was lower than in insects maintained and tested at 23°C. The enzyme activity then increased and was at its maximum in 8 days. They could find no compensation in midgut amylase activity.

The objective of this research was to explore the rate of acclimation, at the locomotory, metabolic, and enzymic levels, of T. confusum, a cold intolerant species, and M. domestica, a cold tolerant species. The rate of acclimation was determined at the locomotory level through measurement of the change in chill-coma temperatures; at the metabolic level through the measurement of the oxygen consumption of the insects after they had been transferred from one temperature to another; and at the enzyme level through the measurement of the activity of the Mg^{++}-activated ATPase of insects.

In this study, the oxygen consumption of T. confusum showed unexpected increases and decreases after transfers from one acclimation temperature to another. M. domestica did not show similar changes in respiration. It is suggested that these changes in oxygen consumption by T. confusum are due to changes in locomotion as a response to the temperature change. It has also been suggested that locomotory acclimation cannot occur until metabolic adjustments have been made. These adjustments perhaps include changes in mitochondrial permeability or number. If the insect is exposed to a low temperature for an extended length of time, changes in the enzyme itself may occur.

MATERIALS AND METHODS

Rearing and Acclimation

The confused flour beetle, T. confusum, was used as the non-cold tolerant insect. T. confusum was reared and maintained on a diet of 50% white flour and 50% corn meal with 5% (by weight) brewer's yeast added as a dietary supplement. The insects were maintained in battery jars or in one pint mason jars at 30°C or 18°C. Edwards (1958) maintained his cultures of T. confusum at 30°C and 18°C for 6 months before testing. The same practice was used here.

The housefly, M. domestica, was used as an example of a cold tolerant insect. One day old adult flies were obtained from the Iowa State University Insectary where they had been reared at 26°C. They were placed in one pint ice cream cartons fitted with nylon net tops and maintained at 30°C or 15°C on a diet of 10% sucrose. Only female flies were used. Thiessen and Mutchmor (1967) acclimated M. domestica for 3 days and found acclimative differences. In this study, four days were allowed for acclimation.

The photoperiod was 12 hours of light and 12 hours of dark per day. The relative humidity was not controlled but varied between 40 and 70%.

To determine the rate of acclimation of two dissimilar insect species it was first necessary to find techniques which could be used with both species. This was true at each of the study levels: locomotory, metabolic, and enzymic.

Change in chill-coma temperature has been used frequently as a measure of acclimation. The usual method for the determination of chill-coma is to watch the insect respond to a mechanical stimulus, such as a

gentle prodding, at a series of low temperatures. After acclimation, the cold-acclimated insect will exhibit movement at a lower temperature than a non-cold acclimated insect. It seemed very likely that the acclimative process involved metabolic adjustments, so a measurement of oxygen consumption was made. In the enzyme study, the magnesium activated ATPases of insect muscle were chosen because, since these enzymes are considered to be one of the chief immediate sources of energy needed for contraction, the rates of acclimation of these enzymes might reasonably be related to the rate of acclimation at the locomotory level.

Locomotion

Tribolium confusum

Preliminary studies indicated that if 30°C acclimated T. confusum adults were exposed to a series of low temperatures, they became immobile after exposure to temperatures below 11°C and would not respond to mechanical stimuli or exhibit spontaneous activity. If 18°C acclimated beetles were exposed to a similar series of low temperatures, they did not become immobile until exposed to a temperature of 9°C. This was the basis of the test for rate of acclimation at the locomotion level. Samples of insects from 30°C and 18°C were removed from the rearing cultures and placed at the opposite temperatures. At intervals after the transfer samples of three insects were removed and tested at 10°C. The testing apparatus consisted of two shell vials, 4.5 cm long with an inside diameter of 1.3 cm, which were permanently fixed through the top of a Plexiglas® box, 8.5 cm long by 5.5 cm wide by 3.5 cm high. The ends of the box had inlet and outlet ports for the cold water that was pumped from a

Blue M refrigerated water bath. The cold water immersed approximately one-half of the length of each vial. In a test the insects were placed in one of the vials which was then covered with a piece of clear plastic. The other vial contained the thermistor used to monitor the test temperature. The insect sample was then examined with a binocular microscope at intervals during a one hour period. The one hour time period was divided into four 900 second intervals, and the number of insects which responded to a gentle prodding or exhibited spontaneous movement was recorded during each time interval.

Musca domestica

The basic technique described for T. confusum was used for M. domestica. However, two changes were necessary because the two species differed in size and cold tolerance. First, preliminary studies indicated that a 30°C acclimated M. domestica placed at 4°C would enter a chill-coma and would not respond to a mechanical stimulus or exhibit spontaneous movement. A 15°C acclimated fly placed at 4°C would not enter chill-coma and would respond to a mechanical stimulus or often, exhibit spontaneous movement. Hence, 4°C was the test temperature used with flies. Second, the cold chamber used for the T. confusum tests was not large enough for use with M. domestica. In place of the box, two 250 ml Erlenmeyer flasks were immersed to their necks in a Blue M refrigerated water bath which was maintained at 4°C. The temperature of the two flasks was monitored with a small surface thermistor taped to the bottom of one of the flasks.

Samples of four insects were placed in the cold chamber and

observed over a one hour time period. The number of insects that would respond to a gentle prodding was determined and recorded during each 900 second interval.

Oxygen Consumption

To determine the rate of acclimation of the respiration rate it was first necessary to establish baseline rates for the two species at their respective acclimation temperatures. Insects were then transferred to higher or lower temperatures and their oxygen consumption measured as they acclimated to the new temperature. For example, 30°C acclimated beetles were transferred to 18°C and 18°C beetles were transferred to 30°C.

Respiration was measured with six Gilmont differential syringe type manometers and a Precision Scientific water bath which was controlled to ± 0.1°C. Samples of insects were placed in the respirometer flasks and allowed to equilibrate for a minimum of one hour before readings were begun. 0.5 ml of 10% KOH was placed in the center well to absorb the CO_2 evolved. After the completion of a test the insects were removed, anesthetized and weighed to the nearest 0.1 mg on a torsion balance. Oxygen consumption was expressed as µl oxygen consumed per mg of insect per hour.

Tribolium confusum

Ten unsexed beetles were used in each test. Tests consisted of no more than six consecutive hourly oxygen determinations. The adults have a life span of about one year (Cotton, 1963) so no attempt was made to use beetles of certain ages. Because of their small size it was not

feasible to restrict the activity of the beetles in the respirometers.

Musca domestica

In each test one female fly was placed in each respirometer. Herms and James (1961) report that the normal life span of the housefly is about two months. In our laboratory the flies maintained at 30°C seldom survived beyond two weeks. Because of this relatively short life span, flies of known age were used in all tests.

Preliminary tests indicated that, if flies were allowed free movement in the flasks, the respiration rates would vary considerably. To reduce movement, flies were confined in small nylon sacs in the respirometers.

Enzyme Study

To determine the rate of acclimation of the enzyme it was first necessary to determine the baseline rates of enzyme activity. Baselines were prepared as follows: T. confusum acclimated at 18°C or 30°C were tested at 18°C and 30°C; M. domestica acclimated at 15°C or 30°C were tested at 15°C and 30°C. Thus, for each species, four sets of baseline values were established. These tests were necessary to show if acclimative differences were present.

Homogenate preparation

Samples of 20 to 50 unsexed T. confusum adults were lightly anesthetized with CO_2 and weighed on a torsion balance to the nearest 0.1 mg. The insects were transferred to a chilled Potter-Elvehjem type glass in glass tissue grinder. A small known amount of cold deionized water was

added to the iced homogenizer and the whole insects were homogenized for 60 revolutions (about five minutes). Studies by Thiessen and Mutchmor (1967) indicated that little increase in homogenate activity occurs after three minutes of grinding. Because of the hard cuticle of our beetles a longer grinding time was used. After completion of the homogenization the sample was stored in an ice bath. A preparation stored in this manner maintained its activity for about eight hours but, in actual practice, no preparation was used after four hours. Final homogenate concentrations were 50 mg per ml.

Because of the larger size of the housefly it was possible to use whole thoraces, rather than whole insects as with T. confusum. Samples of 6 to 8 thoraces were used for each test. The thoraces were obtained by first lightly anesthetizing flies with CO_2 and then rapidly removing the head, abdomen and legs. Each thorax was then transferred immediately to a chilled glass vial and kept on ice until all of the sample was obtained. The sample was then prepared in the same manner as with T. confusum except that the thoraces were homogenized for 48 rather than 60 revolutions. Final homogenate concentrations were 30 mg per ml.

ATPase activity determinations

For the baseline and transfer experiments, ATPase reactions were carried out in tubes suspended in a water bath maintained at 30°C or 18°C for T. confusum, and at 30°C or 15°C for M. domestica. In each test, four 16 ml plastic centrifuge tubes were used. Each tube contained 0.2 ml of 0.02 M $MgCl_2 \cdot 6H_2O$; 1.56 ml of 0.038 M sodium barbital buffer, pH 7.8; and 0.02 ml of 0.02 M ATP as the disodium salt (Nutritional Biochemical

Company). These tubes were equilibrated in the water bath and the reaction was started by the addition of 0.05 ml of homogenate to each of two tubes. The remaining two tubes served as reagent and residual phosphate controls. The usual reaction time was 5 minutes. The reaction was stopped by the addition of 2 ml of 10% trichloroacetic acid, which was also added to the two control tubes. To one of the control tubes 0.05 ml of homogenate was now added. This tube provided a measure of the residual phosphate present in the system. All four tubes were then centrifuged at 0°C and 10,000 rpm (about 13,000 x G) for 8 minutes. The supernatant from each tube was used to make colorimetric phosphate determinations by the method of Fiske and Subbarrow (1925). Each supernatant was placed in a spectrophotometric tube with 1 ml of 2.5% ammonium molybdate and 0.4 ml of aminonaptholsulfonic acid solution. The aminonaptholsulfonic acid solution consisted of 6.0 gm of $Na_2S_2O_5$, 1.2 gm Na_2SO_3 and 0.1 gm 1-amino-2-napthol-4-sulfonic acid. The $Na_2S_2O_5$ and Na_2SO_3 were first dissolved in 50 ml of water and the acid was dissolved in that solution. The tube contents were then diluted to 15 ml with distilled water. Measurements of the percentage transmittance at 600 mu were made on a Coleman Universal spectrophotometer (Model 14) zeroed at 100% transmittance with the reagent blank. The percentage transmittances of the other tubes were then measured within a five minute period. The two readings from the reaction tubes were averaged, and the amount of inorganic phosphate present was calculated through the use of a formula describing a standard calibration curve prepared for the spectrophotometer. The residual phosphate concentration was subtracted, thus giving the total phosphate produced. This value was then converted to µg Phosphate/mg of insect/minute.

Temperature coefficients were calculated using the following form of the Arrhenius equation:

$$\mu = 2\left(\frac{\log_e K_2 - \log_e K_1}{1/T_1 - 1/T_2} \right)$$

where

K_1 = rate of reaction at the absolute temperature, T_1

K_2 = rate of reaction at the absolute temperature, T_2

2 = gas constant (actual value = 1.98).

RESULTS

Locomotion

Figures 1 and 2 represent the transfers of 30°C acclimated insects to 18°C or 15°C and the transfers of 18°C or 15°C acclimated insects to 30°C. Lines of best fit were drawn, by eye, for the first (0-900 second) time interval and the fourth (2700-3600 second) time interval.

In the transfer of warm-acclimated insects to a cold temperature they all are, initially, inactive in the one hour test period. As the insects become acclimated to the cold temperature the gain in cold tolerance is first indicated in the fourth time interval and last in the first time interval. The insects in this transfer are considered acclimated when all the insects are active in the first interval. In the transfer of cold-acclimated insects to a warm temperature, initially, the insects are all active in the one hour test period. As the insects begin to acclimate to the warm temperature they first lose their cold tolerance in the first time interval and finally in the fourth time interval. The insects in this transfer are considered acclimated when the insects show no activity in the fourth time interval.

Figure 1 represents the transfer of 30°C acclimated T. confusum to 18°C and the transfer of 18°C acclimated T. confusum to 30°C. In the transfer of insects from 30°C to 18°C evidence of acclimation is seen as early as 10 hours in the fourth time interval (closed triangles). After about 80 hours at 18°C the insects are all active in the same time interval. Evidence of acclimation in the first time interval (open circles) becomes apparent after about 50 hours and is not completed in over 400 hours. In the transfer of 18°C insects to 30°C evidence of acclimation,

in less than 20 hours, can be seen in both the first time interval and the fourth time interval. Acclimation is complete in about 45 hours in the first interval. The insects in the fourth interval had not completed this acclimation in over 400 hours.

Figure 2 represents the transfer of 30°C acclimated M. domestica to 15°C and the transfer of 15°C acclimated M. domestica to 30°C. In the transfer of 30°C insects to 15°C evidence of acclimation to 15°C is seen as early as 5 hours in the fourth interval. Acclimation is complete in the fourth interval in less than 30 hours. Acclimation in the first interval lags behind that in the fourth interval in a manner similar to that found with T. confusum. Evidence of acclimation is first apparent at about 30 hours and is nearly complete by about 60 hours. In graphing the data obtained from the transfer of 15°C insects to 30°C, no attempt was made to place lines of best fit through points in the first and the fourth time intervals; the lines of best fit essentially overlap in each of the four time intervals. Acclimation is again evident as early as 5 hours and, in this case, is complete in less than 50 hours.

Oxygen Consumption

Average rates of oxygen consumption were determined at 30°C and 18°C for T. confusum and 30°C and 15°C for M. domestica and are given in Table 1.

Figure 1: Rate of locomotory acclimation in T. confusum. Ordinate: Number of active insects. Abscissa: Time, in hours, spent at the indicated transfer temperatures; log-scale. Symbols: Open circles = 0-900 second time interval of the 1 hour test period; closed circles = 900-1800 second time interval; open triangles = 1800-2700 second time interval; closed triangles = 2700-3600 second time interval. Line A fitted to the closed triangles; Line B fitted to the open circles.

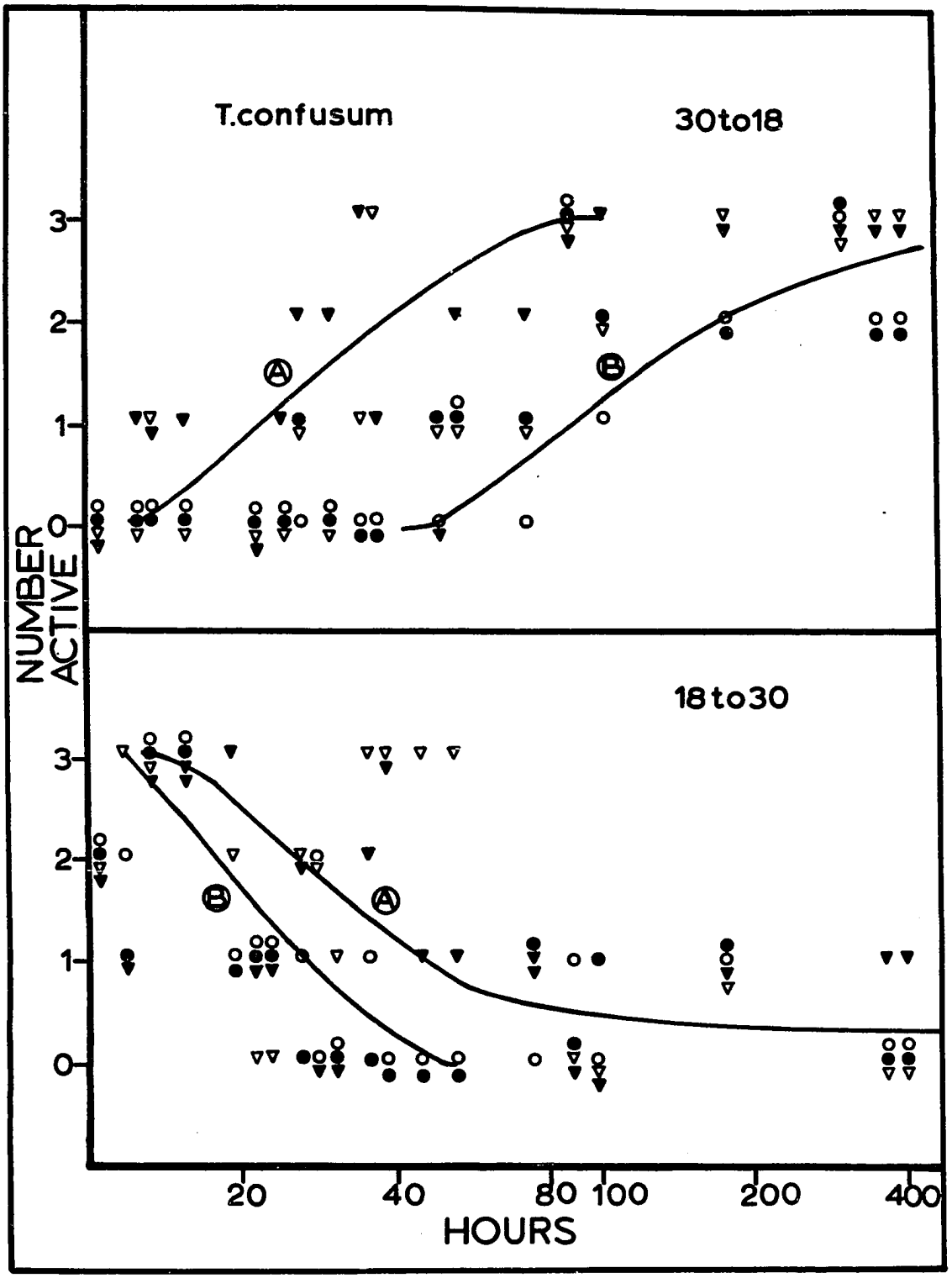

T.confusum
30to18
18to30
NUMBER ACTIVE
0
1
2
3
20
40
80
100
200
400
HOURS
A
B

Figure 2: Rate of locomotory acclimation in M. domestica. Ordinate: Number of active insects. Abscissa: Time, in hours, spent at the indicated transfer temperatures; log-scale. Symbols: Open circles = 0-900 second time interval of the 1 hour test period; closed circles = 900-1800 second time interval; open triangles = 1800-2700 second time interval; closed triangles = 2700-3600 second time interval. Line A fitted to the closed triangles; Line B fitted to the open circles.

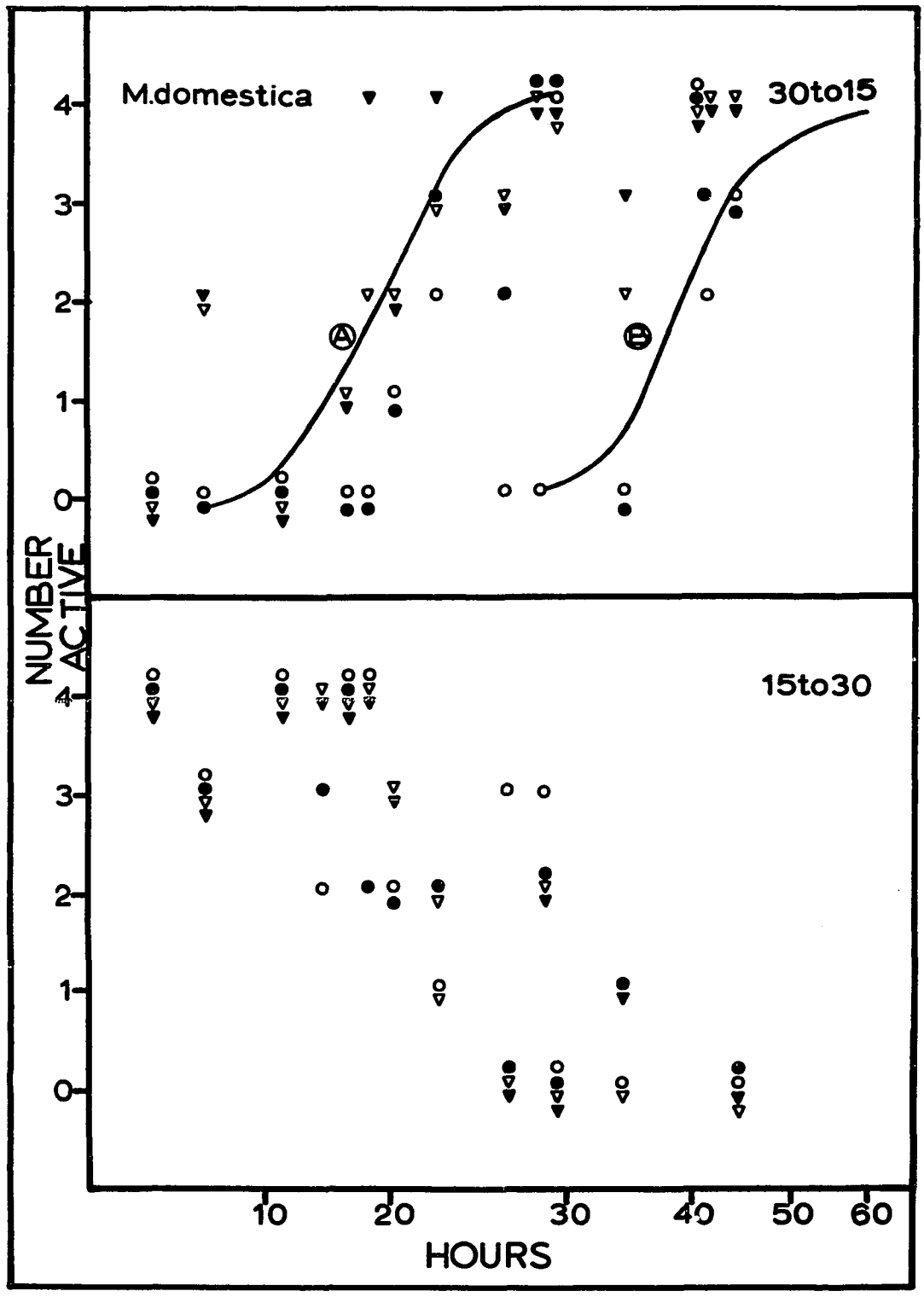

M.domestica
30to15
15to30
NUMBER ACTIVE
HOURS
0
1
2
3
4
10
20
30
40
50
60
A
B

Table 1. Average (µl O_2/mg/hour) values for T. confusum and M. domestica

Species	Acclimation temperature (°C)	Number of hourly readings	Average values ± 1 S.D.
T. confusum	18	68	0.742 ± 0.176
	30	77	2.199 ± 0.476
M. domestica	15	86	0.841 ± 0.338
	30	46	3.060 ± 0.882

The values from Table 1 are indicated in Figures 3 and 4 by horizontal lines labelled 30°C at 30°C and 18°C at 18°C for T. confusum and 30°C at 30°C and 15°C at 15°C for M. domestica. These constitute the oxygen consumption baselines.

Figure 3 represents the measurements of the oxygen consumption of T. confusum after transfer from 30°C to 18°C and from 18°C to 30°C. Each point on the graph is an average of from 4 to 10 hourly readings. Following the transfer of insects from 30°C to 18°C there was an initial increase in the oxygen consumption. This was not the response expected in a transfer of insects from a high to a low temperature. The increase reached a peak after 14 to 18 hours and then consumption began to decrease. Consumption approached the baseline rate established for insects acclimated and tested at 18° in 30 to 50 hours, and reached the baseline about 60 hours after the transfer. The transfer of insects from 18°C to 30°C also produced an unexpected result. In this case there was a decrease in oxygen consumption for the first 30 hours followed by an increase to the baseline rate established for 30°C acclimated insects

tested at 30°C. The baseline was reached in about 80 to 90 hours after the transfer was initiated.

Figure 4 illustrates the oxygen consumption of 15°C and 30°C acclimated M. domestica following their transfer to 30°C and 15°C, respectively. Each point is an average of between 4 and 18 hourly readings. In the transfer of insects from 30°C to 15°C there was an immediate drop in oxygen consumption from the 30°C baseline to the 15°C baseline. No obvious increase in oxygen consumption, similar to that found in T. confusum, occurred. In the transfer of insects from 15°C to 30°C there was a slow increase in oxygen consumption from the 15°C baseline toward the 30°C baseline. The baseline was crossed at about 12 to 15 hours. The oxygen consumption continued to increase in an overshoot and finally reached a maximum at about 25 to 30 hours. A slow decrease in oxygen consumption then took place, until the baseline rate was reached at about 70 to 80 hours.

Enzyme Study

Average values for rate of ATPase activity were determined for 30°C and 18°C acclimated T. confusum and 30°C and 15°C acclimated M. domestica. These rates are given in Table 2.

Figure 3: Rate of acclimation is indicated by measurement of oxygen consumption of T. confusum, Ordinate: Oxygen consumption in µl/mg insect/hour. Abscissa: Time in hours at the indicated transfer temperature. 30 at 30 and 18 at 18 indicate baseline rates.

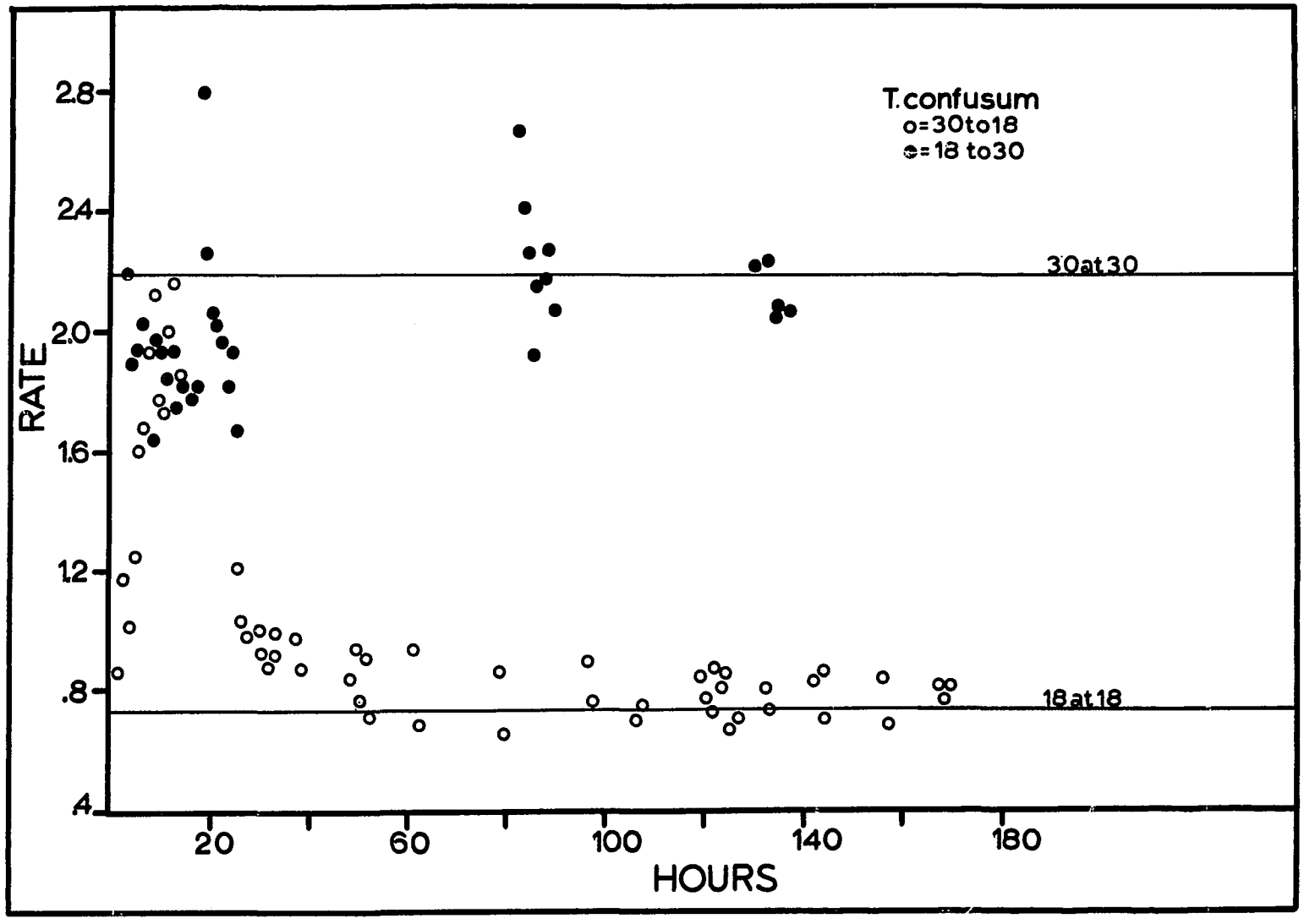

T.confusum
o=30to18
●=18 to30
30at30
18at18
RATE
2.8
2.4
2.0
1.6
1.2
.8
.4
20
60
100
140
180
HOURS

Figure 4: Rate of acclimation is indicated by measurement of oxygen consumption of M. domestica. Ordinate: Oxygen consumption in µl/mg insect/hour. Abscissa: Time in hours at the indicated transfer temperature. 30 at 30 and 15 at 15 indicate baseline rates.

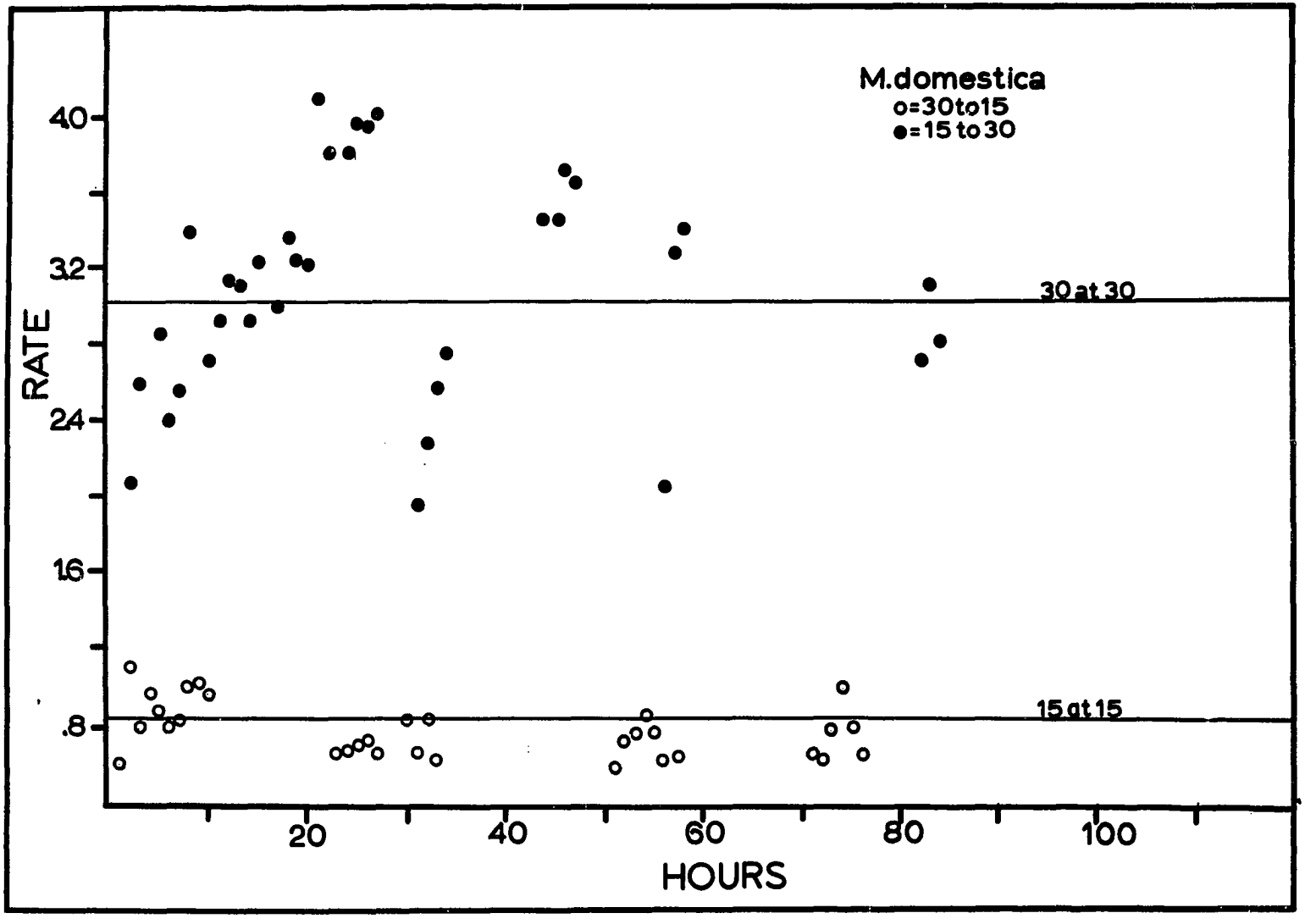

M.domestica
o=30to15
●=15 to30
40
32
24
16
.8
RATE
30 at 30
15 at 15
20
40
60
80
100
HOURS

Table 2. ATPase rates (ugP/mg insect/min) of warm and cold-acclimated T. confusum and M. domestica

Species	Acclimation temperature (°C)	Test temperature (°C)	Number of tests	Average values ± 1 S.D.
T. confusum	18	18	12	0.274 ± 0.062
	18	30	12	1.058 ± 0.234
	30	30	13	0.697 ± 0.098
	30	18	12	0.195 ± 0.082
M. domestica	15	15	18	1.326 ± 0.578
	15	30	21	5.280 ± 0.324
	30	30	15	5.730 ± 0.955
	30	15	13	1.566 ± 0.232

Figure 5 represents the average values for rate of ATPase activity in T. confusum for insects acclimated for 6 months at 18°C and 30°C. It can be seen that cold acclimation resulted in a translation and rotation of the T-R curve to the left in the cold-acclimated insect. It can also be seen that there was little difference between enzyme rates of insects acclimated at 18°C and 30°C and then tested at 18°C. Because of the small difference, no transfer of insects was made, i.e., there was no point in attempting to determine rate of re-acclimation when there was no evidence of acclimation. Insects acclimated to 18°C and 30°C and then tested at 30°C did show a difference in enzyme activity. Because of this difference, insects acclimated at 30°C were transferred to 18°C and then homogenates of these insects were tested at 30°C. As the insects became acclimated to 18°C the enzyme rate was expected to approach the baseline rate previously established for 18°C acclimated insects tested at 30°C.

Figure 6 shows the results of the study of rate of acclimation of ATPase activity in T. confusum. There was an initial decrease in enzyme activity to levels below the 30°C baseline. This reduced rate persisted for about 150 hours. A slow increase in activity was then evident. After the insects had been at the new temperature about 900 hours the enzyme rate had reached the 30°C baseline. A determination was made at 1500 hours (not shown in Figure 6); the rate was still at the 30°C baseline level.

Figure 7 represents the average values for rate of ATPase activity in M. domestica for insects acclimated for 4 to 12 days at 30°C or 15°C. It can be seen that there was not a significant translation or rotation of the T-R curves with either the 30°C or the 15°C acclimated insects. It is also evident that little difference in enzyme rate occurred between flies acclimated at 30°C and 15°C and tested at 30°C and 15°C. Because of the small difference (i.e., little apparent acclimation) no attempt was made to determine the rate of acclimation of ATPase activity in M. domestica.

The data for ATPase activity in these two insects seems to indicate a basic difference in response to temperature after acclimation. To obtain more information about the enzymic response to temperature, rate determinations were carried out at a series of temperatures, extending from near the chill-coma temperatures to about 38°C with homogenates of cold and warm-acclimated insects. The results of these tests are shown in Figure 8. Figure 8 indicates that the general relationships shown in Figures 5 and 7 extend to higher and lower temperatures in both species. But, near the chill-coma temperature differences could be seen in the

Figure 5: ATPase activity of 30°C or 18°C acclimated T. confusum tested at 18°C and 30°C. Ordinate: Enzyme rate in µg P/mg insect/min. Abscissa: Test temperatures in °C. Vertical lines at each test temperature indicate one standard deviation.

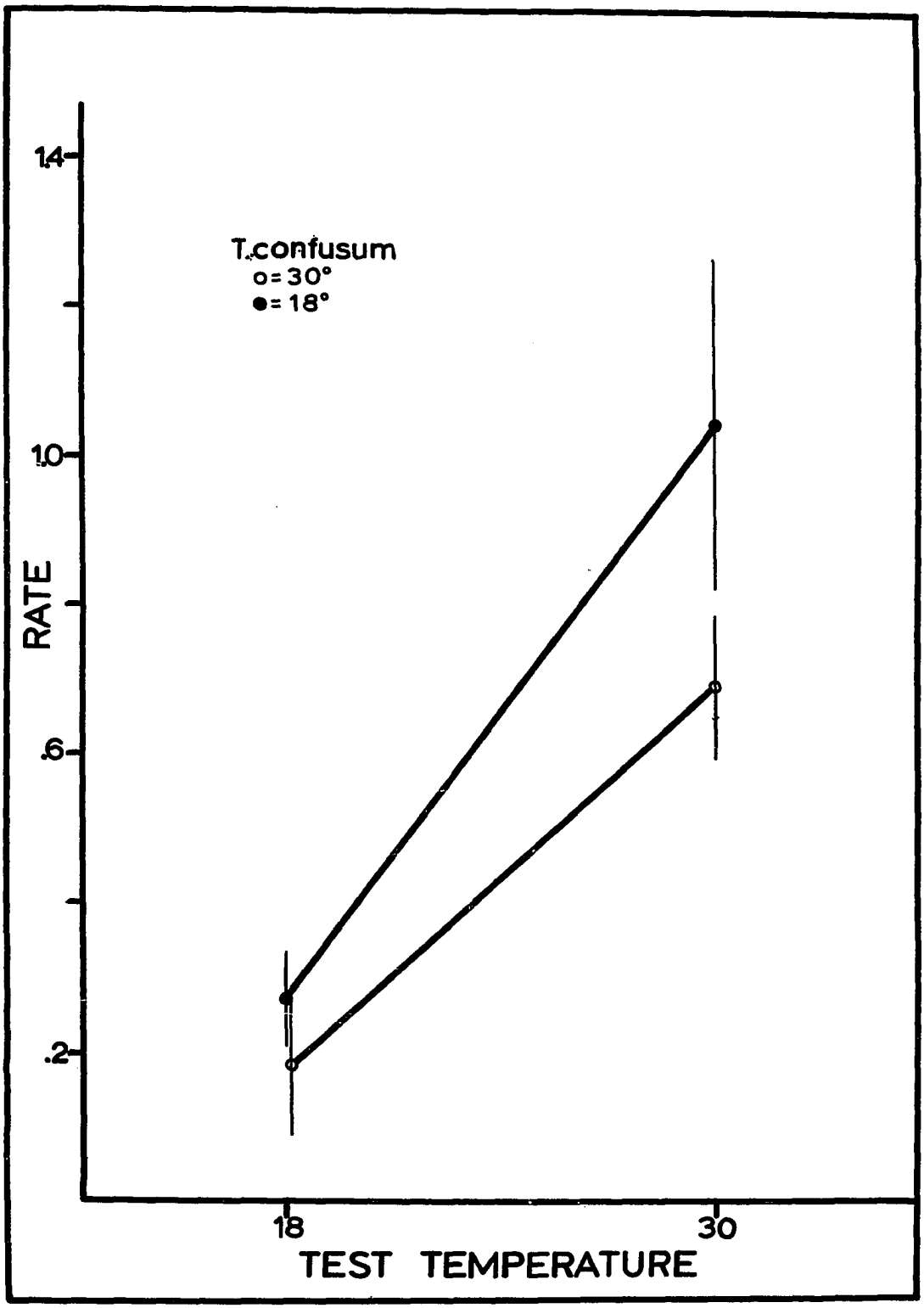

T.confusum
o=30°
●=18°
1.4
1.0
.6
.2
RATE
18
30
TEST TEMPERATURE

Figure 6: Rate of ATPase acclimation in T. confusum. Ordinate: Enzyme rate in µg P/mg insect/min. Abscissa: Acclimation time, in hours, at 18°C; log-scale. 18 at 30 and 30 at 30 indicate baseline rates. Vertical lines at each point indicate one standard deviation.

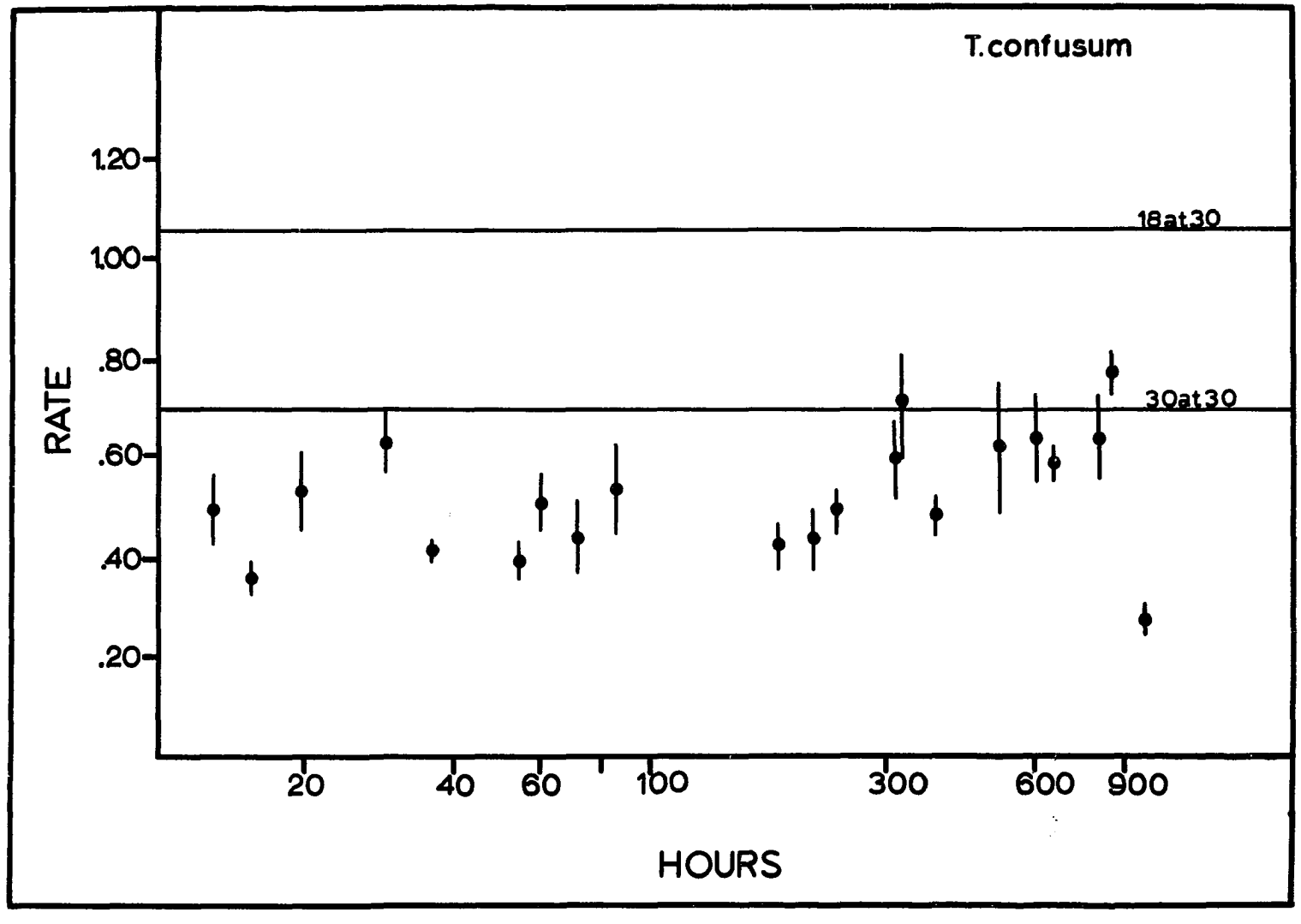
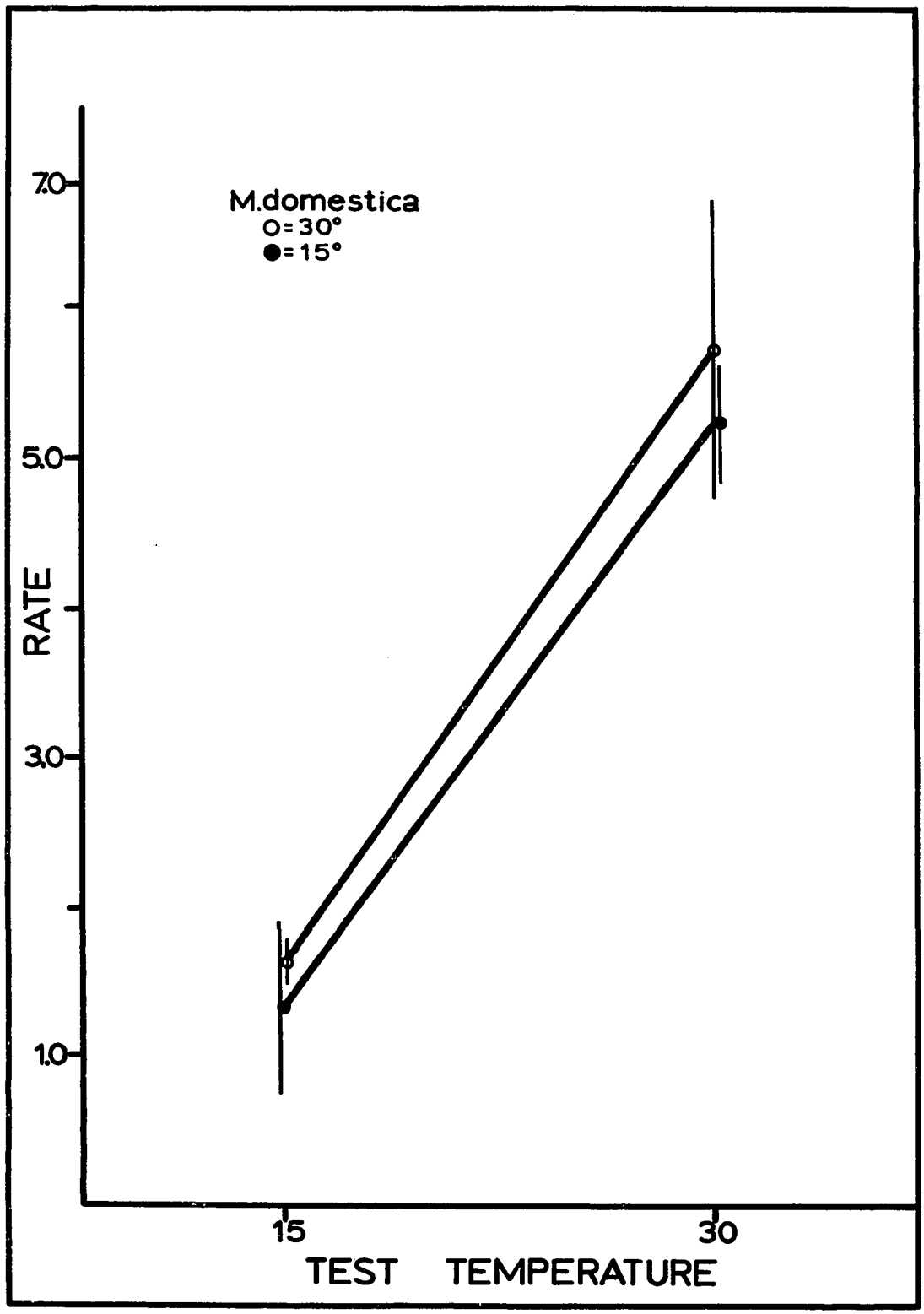

T.confusum
1.20
1.00
.80
.60
.40
.20
RATE
18at30
30at30
20
40
60
100
300
600
900
HOURS

Figure 7: ATPase activity of 30°C and 15°C *M. domestica* tested at 15°C and 30°C. Ordinate: Enzyme rate in µg P/mg insect/min. Abscissa: Test temperatures in °C. Vertical lines at each test temperature indicate one standard deviation.


M.domestica
O=30°
●=15°
7.0
5.0
3.0
1.0
RATE
15
30
TEST TEMPERATURE

pattern of enzyme activity. To define these differences, temperature coefficients were calculated for the temperature range extending from the chill-coma temperature to a temperature 5 degrees higher. The results of the calculations are summarized in Table 3.

Table 3. Temperature coefficients of warm and cold-acclimated *T. confusum* and *M. domestica*

Species	Acclimation temperature (°C)	Temperature range (°C)	Temperature coefficient (cal.)
T. confusum	30	11-16	31,000
	18	11-16	37,000
M. domestica	30	5-10	40,500
	15	5-10	45,000

A comparison of μ-values over similar physiological temperature ranges indicates that the cold-acclimated insects of both species have a higher value than the warm-acclimated insects. The higher μ-values correspond to a steeper slope and represent a greater sensitivity to temperature change. This is the same relationship found in interspecific comparisons (Mutchmor & Richards, 1961).

The results of the present study are summarized in Table 4. The raw data are tabulated in the appendix.

Figure 8: Comparison of the effect of temperature on the rate of ATPase activity in cold and warm-acclimated M. domestica and T. confusum. Ordinate: Natural logarithm of rate (µg P/mg insect/min) of ATPase activity. Abscissa: Reciprocal of absolute temperature (x 10^4). The small numbers above the abscissa are reference temperatures, in ^{o}C.

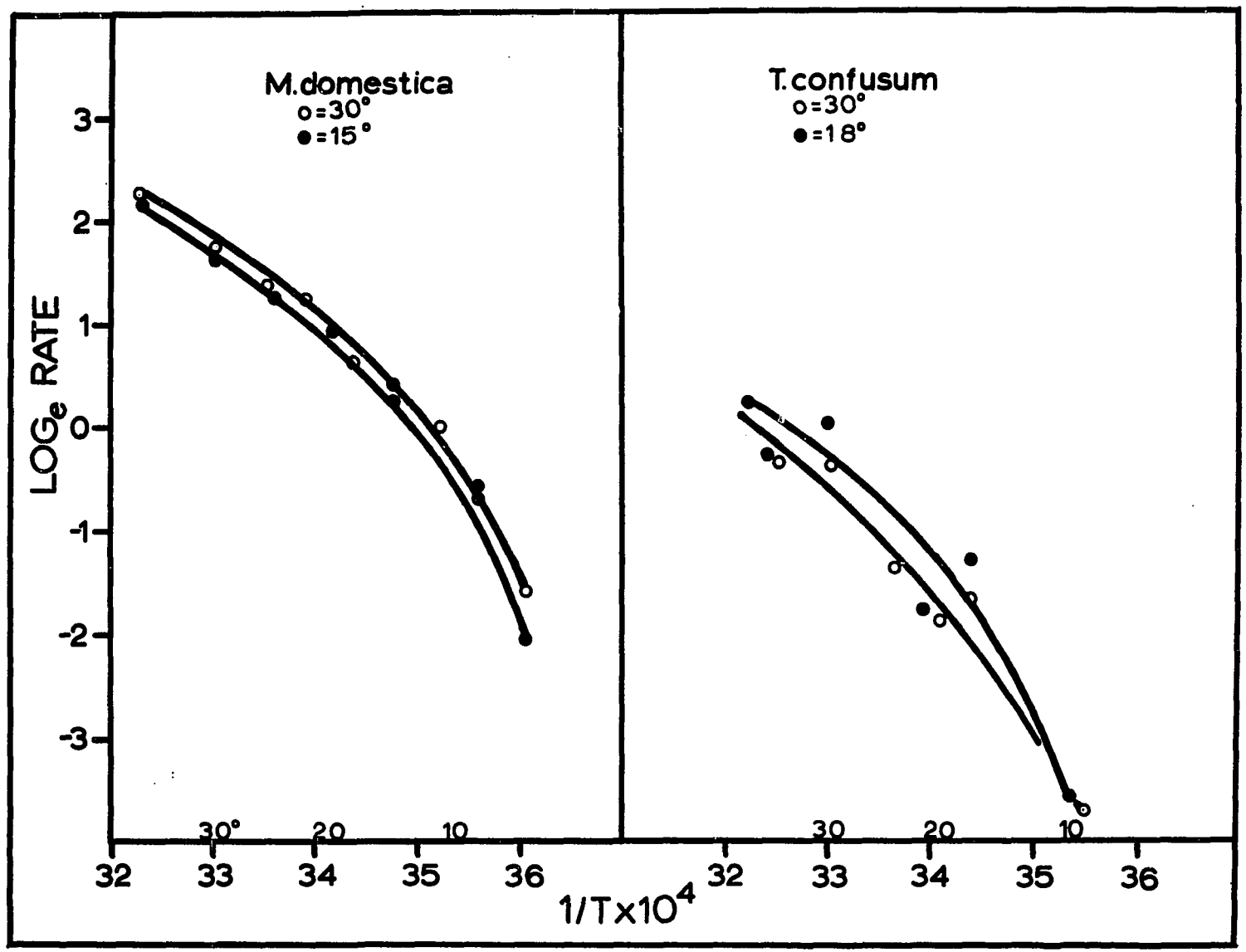

M.domestica
o=30°
●=15°
T.confusum
o=30°
●=18°
LOGe RATE
3
2
1
0
-1
-2
-3
30°
20
10
32
33
34
35
36
1/T×10^4

Table 4. Summary of the rate of temperature acclimation (in hours) of T. confusum and M. domestica with respect to locomotion; oxygen consumption; and ATPase activity

Species	Transfer	Locomotion	Respiration	ATPase
T. confusum	30 to 18	>400	60	>1500
	18 to 30	>400	90	
M. domestica	30 to 15	60	<10	No test
	15 to 30	50	80	

DISCUSSION

Three major types of climate are important in the biology of insects. These are: 1) the tropical, in which the temperature is never low enough to impede insect activity; 2) the continental type, in which summers are essentially tropical and winters are long and cold, and; 3) the constantly cool climate which is characteristic of areas along sea coasts, islands and high mountains in the Tropics (Fairchild, 1969). Those insects which inhabit regions with the continental climate are exposed to wide seasonal variation in temperature and have developed physiological adaptations which allow survival in these temperature extremes. It is well known that, in the continental climate, relatively short-term increases and decreases in temperature also occur. For an insect to be successful in this type of climate it would presumably be useful for it to have the ability to compensate for these short-term temperature fluctuations.

It has already been shown that insects show adaptive differences in chill-coma temperature, respiration and enzyme rates which can be correlated to the normal habitat temperatures usually encountered (see Introduction). To understand the results which were obtained in this study it is important that an examination be made of the normal habitats of the two test insects.

Tribolium confusum is a cold intolerant, long-lived, general feeder on farinaceous foods. It is probably the worst insect pest of prepared cereal foods. It is usually found in granaries, mills, warehouses, and in grain shipments. The insect is essentially a non-flyer; its only means of self-dispersal within the grain or stored-products is by walking. In this

insect's normal habitat the temperature variation is minimal, at least on a short term basis. Cotton (1963) measured the temperature at the center of a 2,740 bushel bin of wheat during a Kansas winter and found that it took two months for the temperature to drop from 25°C to 18°C. The results of the present study indicate that slightly more than 17 days were required for locomotory acclimation to occur. The insect would thus have had ample time to acclimate in conjunction with such slow decreases in temperature.

Storage facilities in colder areas would cool more rapidly than those in Kansas. Watters (1966) reports that the temperature in unheated warehouses in the prairie provinces of Canada fluctuate between zero and 15°C depending on the severity of the outdoor temperatures. Ernst and Mutchmor (1969) acclimated T. confusum to 30°C and 15°C and tested their dispersal ability as a function of temperature. The 30°C acclimated insect showed no dispersal below 10°C, a temperature slightly lower than the chill-coma temperature as determined in this study. The 15°C acclimated insect showed dispersal activity to 5°C. Because bulk stored grain cools slowly and the insect is able to acclimate as the temperature changes, locomotion is possible through two-thirds of the low temperature range normally found in the Canadian grain facilities. Presumably, dispersability following acclimation would be even less limited in the storage facilities in much of the United States.

Work has also been done on the effect of acclimation on temperature preference. Graham (1958) kept T. confusum at 20°C and 27°C for three weeks and determined the temperature preference of the two groups of insects in a temperature gradient. Samples were then moved from 27°C to 20°C and the temperature preference was determined at 1, 2, 4, and 16 days

after transfer to 20°C. Graham's figures indicate that, after 16 days at 20°C, the temperature preference of the transferred insects is the same as the control insects held at 20°C.

Aquatic poikilotherms inhabit an environment which is similar to that of T. confusum with respect to thermal stability. Spoor (1955) determined the loss and gain of heat tolerance in the freshwater crayfish, Astacus. He transferred crayfish from a maintenance temperature (23-24°C) to 4°C and measured the loss of heat tolerance. After 2½ days at 4°C there was no change in the heat tolerance. Gradually the heat tolerance decreased until at 16 days the crayfish responded as a 4°C animal. He also transferred crayfish from the maintenance temperature to 12°C and found that 14 days were necessary for the crayfish to attain the same heat tolerance as 12°C crayfish. In another study, McLeese (1956), using the American Lobster, Homarus americanus, found that lobsters transferred from 14.5°C to 23°C required approximately 22 days to re-acclimate.

M. domestica is a relatively short-lived, cold tolerant insect with cosmopolitan distribution. If acclimation to temperature is to be of adaptive value to this insect it must occur in a relatively short time. In the present study it was found that locomotory acclimation to cold takes approximately 60 hours or 2½ days. This is approximately 8% of the normal life span of the female. A lag period of 30 hours occurs, after the change in temperature from 30°C to 15°C, before locomotory acclimative changes can be detected. The lag period may be due to metabolic acclimations which must occur before locomotory acclimative changes can be expressed. It may also be of importance in reducing the effect of very short term environmental temperature changes.

There have been no other studies on the rate of locomotory acclimation with M. domestica, but several studies with physiologically similar (with respect to cold tolerance) species have shown similar rates of acclimation. Mutchmor and Richards (1961), Thiessen and Mutchmor (1967), and Anderson and Mutchmor (1968) acclimated the American cockroach, P. americana, for a minimum of three days and found three to five degrees difference in chill-coma temperature. Thiessen and Mutchmor (1967) also acclimated the housefly and could show chill-coma differences after three days. As already noted in the present study, 60 hours or 2½ days were required for locomotory acclimation.

No studies have been done with M. domestica to show the effect of temperature acclimation on temperature preference. However, Nieschulz (1935) determined the temperature preference of M. domestica and found a wide range of thermal preference, from 20°C to 40°C with the greatest number of individuals preferring temperatures above 35°C. The distribution was skewed to the left, however, with some individuals preferring temperatures as low as 15°C.

Oxygen Consumption

There is little information relating rate of acclimation and oxygen consumption in insects. Edwards (1958) determined the oxygen consumption of T. confusum after a transfer from 30°C to 18°C and from 30°C to 38°C. He found, following the 30°C to 18°C transfer, that the insects reached the rate characteristic of 18°C acclimated insects tested at 18°C in 24 hours. He concluded that no respiratory compensation occurred and that only a "passive" lowering of respiration had taken place. From the information in his paper, however, it is evident that the oxygen consumption

rates were determined at one day intervals. In the present study the oxygen consumption was determined at one hour intervals during the first 13 hours after transfer, and the results obtained differ from those of Edwards. The first 24 hours after a transfer from 30°C to 18°C does not result in a "passive" lowering of respiration. Instead, there is a substantial increase in respiration. This increase may be due to a respiratory compensation in response to the decrease in temperature, or it may be a behavioral compensation which has influenced the respiration of the insect. It was not possible to measure accurately any increase, per se, in locomotory activity with the present experimental equipment. It is well known, however, that activity is a major cause of increased oxygen consumption (Prosser, 1961). An increase in activity after a transfer from a warm to a cooler environment would be a distinct advantage to a cold intolerant insect like T. confusum. As the environmental temperature decreased the increase in the insect's activity would give it at least some chance to move away from the cooler area. Edwards reported, for the transfer from 30°C to 38°C, an overshoot which lasted for 2 to 3 days, followed by a decrease in respiration. In the present study the transfer from 18°C to 30°C resulted in a decrease in respiration which lasted for approximately 24 hours. There then occurred a slow increase which required 80 to 90 hours to reach the 30°C baseline. It is not possible to compare in detail the results of Edward's transfer of 30°C acclimated insects to 38°C with those of the present study because of the differences in temperature ranges used. Edwards reports that the 38°C insects exhibited poor motor coordination, indicating a possible deleterious effect of the high temperature. The transfer of insects from 18°C to 30°C does not

stress the insect but actually represents a return of the insect to a temperature which is nearer to its optimum. At present, it is not possible to ascribe any adaptive significance to the decrease in respiration found in the transfer of insects from 18°C to 30°C.

Figures 3 and 4 reveal that it is difficult to show acclimative differences with respect to oxygen consumption. In order to show such differences it is necessary to determine the rate of oxygen consumption of two differently acclimated insects at a series of temperatures. But, the present results suggest that the insect starts to acclimate to the test temperature during the test. Naturally, this tends to obscure the results and makes interpretations difficult at best.

Enzyme Study

It has been reported that the usual manifestation of cold acclimation or adaptation in poikilotherms is translation and rotation of T-R curves, resulting in a decreased slope (Prosser, 1961). The interpretation, in terms of adaptive advantage, is that the reduced slope would allow the cold-acclimated poikilotherm to be less temperature dependent. An alternative response is translation and rotation of the T-R curve in the opposite direction, resulting in an increase in the slope and, therefore, making the animal more temperature sensitive. Scholander, et al. (1953) measured the oxygen consumption of arctic and tropical poikilotherms and reported increases in slope in molluscs, spiders, and insects. They did not consider these changes to be adaptive; they considered only reduced temperature dependence to have adaptive value. Mutchmor and Richards (1961) reported greater slopes in cold tolerant insects than in cold intolerant insects.

Figures 5 and 7 indicate the ATPase activity of T. confusum and M. domestica through the range of 15°C or 18°C to 30°C. T. confusum shows a translation and rotation to the left, indicating that the process has become more sensitive to temperature changes. Through this temperature range a small increase in temperature would allow a substantial increase in ATPase rate. M. domestica shows a response opposite to that of T. confusum. The warm-acclimated flies actually exhibited greater rates of ATPase activity at given temperatures. There was also a slight decrease in μ-value for the cold-acclimated flies. In this respect, at least, the fly was like many other cold-acclimated poikilotherms.

To obtain more information about the ATPase activity throughout the normal range of habitat temperatures of the test insects, a series of enzyme rate determinations was made at temperatures extending from the chill-coma temperature of each insect to 38°C. As indicated previously, these results are shown in Figure 8. To compare the ATPase rates through similar physiological temperatures the μ-values were calculated in the range from the chill-coma temperature to the chill-coma temperature plus 5°C. In this low temperature range the relationships for T. confusum found in the 18°C to 30°C test range persist, with the cold-acclimated insects having higher μ-values. But, with M. domestica, relationships found in the 15°C to 30°C temperature range are reversed in the lower range. At the low temperatures the cold-acclimated fly has a substantially higher μ-value, thus indicating a greater temperature dependence.

The results found here agree with the interspecific differences shown by Mutchmor and Richards (1961). The usual interpretation of the increased slope at low temperature is that slight increases in temperature

would allow substantial increases in ATPase activity and, therefore, perhaps allow muscular activity. The differences in μ-values are also reflected in gross observations of the insects in chill-coma as they are slowly warmed. M. domestica recovers from chill-coma more quickly than T. confusum, and its movements become very vigorous far more rapidly than do those of T. confusum. The higher μ-values in the cold-acclimated insects contradict the results obtained by Thiessen and Mutchmor (1967). In fact, throughout this study, the ATPase values have been substantially higher than those obtained by Thiessen and Mutchmor. This may be due to improved homogenate preparation or other unknown factors.

The study of rate of acclimation of the ATPase activity in T. confusum and the absence of any difference in ATPase activity in the normal temperature range for M. domestica are perplexing. With M. domestica no substantial difference in ATPase activity could be found at the 15°C and 30°C test temperatures. It can be assumed that through this temperature range there is ample enzyme activity to serve the needs of the fly irrespective of temperature, and it is only at the lower temperatures that differences can be seen and are important. But from the locomotion study it is evident that approximately 60 hours are needed for acclimation. The study of rate of enzymic acclimation in T. confusum indicated that at least 900 hours were needed for acclimation whereas locomotory acclimation was complete in slightly more than 400 hours.

Several recent papers by Newell and his associates (1966, 1967) may aid in the interpretation of the present results. It has been assumed for many years that, as the temperature of the environment increases, the oxygen consumption of poikilotherms exposed to these changes would also

increase. Newell (1966) determined the oxygen consumption of the cockle, Cardium edule, through a series of four temperatures ranging from 5°C to 20°C, and correlated the respiratory rate with observations of the activity of the animal. He found that the oxygen consumption, which was measured while the animal was active, increased with temperature. However, the oxygen consumption of the quiescent animal did not increase with temperature but, instead, showed an independence of temperature. Newell and Northcroft (1967) obtained a similar result with 5 other intertidal animals. A study was also done to establish whether similar temperature relationships could be shown at the mitochondrial level (Newell, 1966). Intact mitochondria were isolated from a series of five poikilotherms from habitats having different normal temperatures. They ranged from the skate, Raia sp., which would seldom experience temperatures above 10°C, to the locust, Schistocera gregaria, which has an optimum environmental temperature of 35°C. The oxidation of succinate and pyruvate was measured at a series of temperatures. The results produced a curve of shallow slope followed by a rapid increase in respiration. In each case, the point of inflection corresponded to the normal habitat temperature of the animal. The area of shallow slope in each case had a Q_{10} of 1.3. A similar study by Newell and Walkey (1966), using rabbit and cow mitochondria, gave the same results.

The results obtained by Newell and his associates (1966, 1967) provides support for the idea, already expressed in the Discussion, that the initial changes in oxygen consumption obtained in the present study may be due to behavioral rather than metabolic responses to the temperature. It is important to note that, in Newell's study, intact mitochondria were

prepared and that low Q_{10} values were found at test temperatures below the normal habitat temperatures. Low Q_{10} values indicate that a physical rather than a chemical action is important. For example, the metabolic activity of the mitochondria may be determined by the rate of a physical phenomenon, such as diffusive transfer of a substrate into the organelles. If this is limiting in acclimation it could be overcome by a change in mitochondrial permeability or an increase in mitochondrial number. Either of these changes would, in effect, increase the surface available for acquisition of substrate.

Evidence is available for both possibilities. Kennedy and Nayler (1965) have shown seasonal variation in the activity of a membrane bound, Na^{+}-K^{+} activated, Mg^{++} dependent ATPase in toad cardiac muscle. The enzyme showed its greatest activity in the summer and its lowest activity in the winter. Bowler and Duncan (1967) have shown that a Na^{+}-K^{+} activated, Mg^{++} dependent ATPase is associated with cation transport across membranes while another ATPase, which is Mg^{++} activated, controls passive permeability. They believe a failure of the Na^{+}-K^{+} activated, Mg^{++} dependent ATPase during exposure to high temperatures in involved in the heat death of the crayfish, Astacus. There is also some evidence for increased numbers of mitochondria with cold acclimation. Thiessen and Mutchmor (1967) reported an increase in mitochondria number in M. domestica and P. americana after cold acclimation. However, Staszak (1968) studied the effect of temperature acclimation on the mealworm, T. molitor, and could find no difference in number with acclimation in that species.

Any changes in mitochondrial membrane permeability or number would be masked in the present study because ruptured mitochondria rather than

intact mitochondria were used. This may explain the great time difference found in T. confusum with respect to locomotory and enzymic acclimation. Acclimation in the locomotory sense may have been completed through changes in mitochondrial properties or number which could not be measured with the present technique. Perhaps it is only after prolonged cold exposure that actual changes in the enzyme itself occurs.

Possible changes which may occur in enzymes with cold acclimation are not well defined at this time. However, Hochachka (1967) reports the possibility that isozymes may be important in cold acclimation in the common goldfish, Carassius auratus. Under conditions of cold acclimation, five lactic dehydrogenase (LDH) isozymes were found. In warm-acclimated goldfish, four LDH isozymes were found, and often only two of the five forms found after cold acclimation were present. Also pertinent to the present study is a paper by Mills and Cochran (1967). They found four different ATPases in the thoracic muscles of the American cockroach, P. americana, each showing a different temperature tolerance. They held homogenates at 0°C for 2, 4, and 6 hours and found that ATPase IV lost 24, 67, and 100% of its activity after exposure to the low temperature for the three time intervals. ATPase II showed no loss in activity during the same low temperature exposure. The other two ATPases were intermediate in loss of activity.

The events which occur upon transfer of an insect from one temperature to another may be explained in the following manner. Initially there is a behavioral response which may cause an increase in the oxygen consumption during the first hours after the transfer, as was shown with T. confusum. This is followed by an extended time period, the duration of

which is species dependent, when possible changes in the mitochondria membranes or other physical processes occur. The biochemical events which occur during this period in various animals have been reviewed by Hochachka (1967) and Rao (1967). After these adjustments have been completed the insect is acclimated, in the locomotory sense, to the new temperature. If the temperature change persists for a relatively long period, perhaps changes occur in the enzyme itself. Thus, an acclimative process that proceeds from the metabolic and locomotory levels and ends with changes at the enzymic level, would be consistent with the results of this study. Furthermore, if one may say so from a rather limited number of species, the greatest rates of acclimation are found in species that carry out their life processes in highly variable environments. Other species, functioning in thermally stable habitats, acclimate slowly.

SUMMARY

The results of the present research indicate that:

1) Locomotory acclimation in T. confusum is not complete in 400 hours. Locomotory acclimation in M. domestica, after transfer from 30°C to 15°C, is complete in 60 hours. Acclimation after transfer from 15°C to 30°C is complete in about 50 hours.

2) After transfer of 30°C acclimated T. confusum to 18°C, oxygen consumption increases to a maximum in about 18 hours. Consumption then decreases to basal values in about 60 hours. The opposite transfer, of 18°C acclimated beetles to 30°C, results in a decrease in oxygen consumption which lasts for about 40 hours, followed by an increase that reaches basal values in 90 hours. Transfer of 30°C acclimated M. domestica to 15°C results in an immediate decrease in oxygen consumption to levels typical of 15°C flies tested at 15°C. No increase in oxygen consumption, as seen with T. confusum, was evident. The opposite transfer, of 15°C acclimated flies to 30°C, resulted in an overshoot that reached a maximum after 30 hours and then declined to basal values in about 80 hours.

3) Cold acclimation of T. confusum leads to a translation and rotation of the ATPase T-R curve. However, the curves for warm and cold-acclimated beetles intersect near the chill-coma temperatures. ATPase T-R curves for cold-acclimated M. domestica do not show a substantial translation. ATPase μ-values for the temperature range extending from the chill-coma temperature to a temperature 5 degrees higher are greater in cold-acclimated than in warm-acclimated insects of both species. This indicates a greater temperature sensitivity at the lower temperatures.

4) Transfer of 30°C acclimated T. confusum to 18°C leads to a decrease in ATPase activity at 30°C which lasts 150 hours. This decrease is followed by a slow increase in ATPase activity. Enzymatically the insect has not completed acclimation to 18°C after more than 900 hours at 18°C.

The unexpected increases and decreases in O_2 consumption by T. confusum, after transfer from one temperature to another, are discussed and compared with the absence of such changes in M. domestica. It is suggested that these changes in O_2 consumption by T. confusum are due to changes in locomotion as a response to the temperature change. It has

also been suggested that locomotory acclimation cannot occur until metabolic adjustments have been made. These adjustments perhaps include changes in mitochondrial permeability or number. If the insect is exposed to a low temperature for an extended length of time, changes in the enzyme itself may occur.

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APPENDIX

The following pages include the data for the locomotion, oxygen consumption, and enzyme rate studies for T. confusum and M. domestica. Table 5 includes the raw data for the locomotion study. The numbers represent the number of insects active at 10°C for T. confusum and 4°C for M. domestica in each of the four time periods of the one hour test period. Table 6 includes the raw values for the oxygen consumption baseline determinations. Table 7 includes the number of determinations and average values for each hour tested in the transfer of warm-acclimated insects to the cold temperature and cold-acclimated insects to the warm temperature. Table 8 includes the raw data for ATPase baseline determinations. Table 9 includes the raw data for the transfer of 30°C acclimated T. confusum to 18°C and then testing homogenates at 30°C. Table 10 includes the average values for the ATPase determinations at a series of temperatures.

Table 5. Locomotion study of T. confusum and M. domestica

Transfer	Hours after transfer	Time interval of one hour test period 0-900	900-1800	1800-2700	2700-3600
30 to 18°C (T. confusum)	1	0	0	0	0
	6	0	0	0	1
	7	0	0	1	1
	11	0	0	0	1
	21	0	0	0	0
	24	0	0	0	1
	26	0	1	1	2
	29	0	1	1	2
	30	0	0	0	2
	35	0	0	1	3
	37	0	0	3	1
	48	0	1	1	0

Table 5. (Continued)

Transfer	Hours after transfer	Time interval of one hour test period 0-900	900-1800	1800-2700	2700-3600
30 to 18°C	53	1	1	1	2
	70	0	1	1	2
	84	3	3	3	3
	100	1	2	2	3
	172	2	2	3	3
	342	2	2	3	3
	383	2	2	3	3
18 to 30°C	1	2	2	2	2
	4	2	1	3	1
	7	3	3	3	3
	11	3	3	3	3
	19	1	1	2	1
	21	1	0	1	1
	23	1	1	0	1
	26	1	0	2	2
	28	2	0	2	0
	30	0	0	0	1
	35	0	1	3	2
	37	0	0	3	3
	46	0	0	3	1
	53	0	0	3	1
	73	0	1	1	1
	89	1	0	0	0
	97	0	1	0	0
	173	1	1	1	1
	355	0	0	0	1
	378	0	0	0	1
30 to 15°C	3	0	0	0	0
(*M. domestica*)	6	0	0	2	2
	11	0	0	0	0
	16	0	0	1	1
	18	0	0	2	4
	20	1	1	2	2
	22	2	3	3	4
	26	0	2	3	3
	28	0	4	4	4
	29	4	4	4	4
	34	0	0	2	3
	40	3	3	4	4
	41	4	4	4	4
	44	2	3	4	4

Table 5. (Continued)

Transfer	Hours after transfer	Time interval of one hour test period 0-900	900-1800	1800-2700	2700-3600
15 to 30°C	3	4	4	4	4
	6	3	3	3	3
	11	4	4	4	4
	14	2	3	4	4
	16	4	4	4	4
	18	4	2	4	4
	20	2	2	3	3
	22	1	2	2	1
	26	3	0	0	0
	28	3	2	2	2
	29	0	0	0	0
	34	0	1	0	1
	44	0	0	0	0

Table 6. Raw values for the oxygen consumption baseline determinations

Species	Acclimation temperature (°C)	Test temperature (°C)	Oxygen consumption values (ul O_2/mg insect/hour)		
T. confusum	18	18	0.977	0.857	0.792
			1.087	0.785	0.438
			0.927	0.888	0.655
			0.742	0.718	0.387
			0.970	1.158	0.720
			0.798	0.806	0.467
			1.110	0.892	0.694
			1.072	0.928	0.445
			0.876	0.834	0.731
			0.940	1.325	0.514
			1.060	0.983	0.610
			0.652	0.533	0.535
			0.845	0.779	1.115
			0.749	0.909	0.552
			0.749	0.718	0.683
			0.737	0.778	0.738
			0.913	0.612	0.705
			0.523	0.721	0.568
			0.644	0.489	0.658
			0.313	0.877	0.458
			0.798	0.239	0.800
			0.342	0.808	0.313
			0.618	0.768	
T. confusum	30	30	2.354	2.446	1.890
			2.142	2.384	2.008
			2.061	2.612	1.910
			2.158	2.022	2.081
			2.036	1.991	2.215
			1.903	2.169	1.809
			2.147	1.701	1.849
			2.620	2.837	2.835
			2.380	2.573	2.548
			1.948	2.224	2.296
			2.009	2.253	2.017
			1.684	2.581	2.330
			2.135	2.106	1.891
			1.945	1.756	1.865
			2.068	2.562	2.528
			2.179	2.638	2.157
			2.289	2.425	2.481

Table 6. (Continued)

Species	Acclimation temperature (°C)	Test temperature (°C)	Oxygen consumption values (ul O_2/mg insect/hour)		
T. confusum	30	30	2.026	2.375	2.290
			1.787	2.750	2.828
			2.008	2.300	2.286
			1.679	1.875	2.186
			2.544	2.192	2.673
			2.404	2.292	2.458
			2.832	2.338	2.134
			2.229	2.376	2.388
			2.015	2.096	
M. domestica	15	15	0.604	0.449	0.895
			0.992	0.499	0.653
			1.080	0.640	0.811
			1.170	0.995	1.121
			0.973	0.726	0.501
			0.693	1.200	0.666
			1.080	1.400	0.455
			1.250	1.336	0.487
			0.639	0.149	0.584
			1.420	0.548	0.729
			1.810	0.750	0.433
			0.827	1.572	0.481
			0.693	1.030	0.861
			1.410	1.052	0.750
			0.990	1.041	0.428
			1.150	0.652	0.579
			0.778	0.679	0.759
			0.737	0.696	0.352
			0.691	0.845	0.475
			0.950	0.671	0.673
			0.939	0.761	1.200
			1.120	1.020	0.491
			0.945	1.300	0.605
			0.657	0.632	2.190
			0.501	1.400	0.666
			1.140	0.751	0.630
			0.636	0.839	0.649
			0.649	0.444	0.694
			0.570	0.883	0.718
			0.905		

Table 6. (Continued)

Species	Acclimation temperature (°C)	Test temperature (°C)	Oxygen consumption values (µl O_2/mg insect/hour)		
M. domestica	30	30	3.030	2.880	3.870
			4.250	3.000	5.250
			3.010	2.490	2.830
			2.830	2.930	2.900
			3.400	2.340	3.470
			2.540	3.320	2.610
			2.910	2.860	2.460
			3.040	2.150	3.090
			3.380	3.290	3.700
			4.220	3.530	2.570
			3.810	3.640	2.890
			3.980	3.240	2.610
			2.970	2.170	5.180
			2.150	1.180	3.480
			4.340	1.770	2.670

Table 7. Oxygen consumption values for the transfer of warm-acclimated insects to a cold temperature and cold-acclimated insects to a warm temperature

Species	Transfer (°C)	Hours after transfer	Number of hourly readings	Average (ul O_2/mg insect/hour)
T. confusum	30 to 18	1	4	0.870
		2	8	1.194
		3	8	1.021
		4	8	1.249
		5	6	1.603
		6	4	1.685
		7	4	1.933
		8	4	2.130
		9	4	1.774
		10	7	1.747
		11	3	2.019
		12	4	2.164
		13	6	1.852
		25	8	1.209
		26	8	1.049
		27	4	0.993
		29	8	1.009
		30	8	0.943
		31	4	0.895
		32	4	0.917
		33	4	1.004
		34	4	0.921
		37	4	0.998
		38	4	0.885
		48	4	0.853
		49	8	0.948
		50	8	0.771
		51	4	0.920
		52	4	0.723
		61	4	0.972
		62	4	0.674
		78	4	0.866
		79	4	0.662
		96	4	0.935
		97	4	0.771
		106	4	0.708
		107	4	0.753
		119	4	0.850
		120	7	0.771
		121	7	0.732
		122	3	0.886
		123	3	0.804

Table 7. (Continued)

Species	Transfer (°C)	Hours after transfer	Number of hourly readings	Average (µl O_2/mg insect/hour)
T. confusum	30 to 18	124	3	0.868
		125	3	0.690
		126	3	0.692
		132	4	0.812
		133	4	0.743
		144	4	0.836
		145	4	0.854
		146	4	0.700
		156	4	0.842
		157	4	0.679
		167	4	0.856
		168	4	0.767
		169	4	0.818
T. confusum	18 to 30	2	8	2.285
		3	8	2.206
		4	4	1.907
		5	4	1.956
		6	4	2.072
		8	4	1.642
		9	4	1.973
		10	4	1.936
		11	8	1.854
		12	8	1.943
		13	4	1.755
		14	4	1.834
		15	4	1.865
		16	4	1.782
		17	4	1.822
		18	4	2.810
		19	4	2.294
		20	5	2.060
		21	9	2.010
		22	8	1.972
		23	9	1.828
		24	10	1.930
		25	4	1.675
		82	6	2.674
		83	6	2.418
		84	6	2.272
		85	6	1.921
		86	6	2.150
		87	6	2.179
		88	6	2.264

Table 7. (Continued)

Species	Transfer (°C)	Hours after transfer	Number of hourly readings	Average (µl O_2/mg insect/hour)
T. confusum	18 to 30	89	6	2.073
		130	6	2.217
		132	6	2.235
		135	6	2.048
		136	6	2.099
		137	6	2.091
M. domestica	30 to 15	1	2	0.638
		2	9	1.110
		3	3	0.811
		4	7	0.973
		5	13	0.872
		6	17	0.822
		7	12	0.834
		8	10	1.000
		9	6	1.050
		10	6	0.966
		13	6	1.290
		23	6	0.664
		24	6	0.671
		25	6	0.700
		26	6	0.736
		27	6	0.684
		30	4	0.828
		31	5	0.674
		32	6	0.844
		33	5	0.628
		51	4	0.591
		52	6	0.723
		53	6	0.746
		54	6	0.848
		55	6	0.775
		56	6	0.615
		57	5	0.630
		71	6	0.651
		72	6	0.637
		73	6	0.785
		74	6	0.986
		75	6	0.782
		76	6	0.653
M. domestica	15 to 30	2	17	2.037
		3	18	2.531
		4	18	2.940

Table 7. (Continued)

Species	Transfer (°C)	Hours after transfer	Number of hourly readings	Average (µl O_2/mg insect/hour)
M. domestica	15 to 30	5	10	2.751
		6	14	2.319
		7	18	2.477
		8	12	3.285
		9	4	1.553
		10	11	2.620
		11	12	2.847
		12	12	3.030
		13	12	2.991
		14	12	2.814
		15	5	3.127
		17	5	2.893
		18	6	3.244
		19	8	3.135
		20	4	3.129
		21	5	3.996
		22	5	3.650
		24	6	3.653
		25	6	3.752
		26	6	3.818
		27	6	3.905
		31	6	1.889
		32	6	2.246
		33	6	2.481
		34	6	2.664
		44	6	3.371
		45	6	3.320
		46	6	3.587
		47	6	3.502
		56	6	2.074
		57	6	3.146
		58	6	3.270
		82	6	2.631
		83	6	2.975
		84	6	2.711

Table 8. Raw data for ATPase baseline determinations (μgP/mg insect/min)

Species	Test combinations (°C)			
T. confusum	18 at 18	30 at 30	30 at 18	18 at 30
	0.394	0.700	0.212	1.280
	0.297	0.580	0.275	1.560
	0.193	0.810	0.132	1.267
	0.326	0.604	0.143	1.092
	0.356	0.613	0.235	0.820
	0.260	0.738	0.315	1.110
	0.260	0.746	0.331	0.986
	0.225	0.804	0.201	0.999
	0.296	0.873	0.137	1.065
	0.250	0.588	0.152	1.010
	0.204	0.600	0.107	0.832
	0.223	0.756	0.106	0.685
		0.649		
M. domestica	15 at 15	30 at 30	30 at 15	15 at 30
	1.212	3.980	2.065	5.640
	1.148	4.170	1.726	5.750
	1.520	4.210	1.800	5.570
	1.855	6.850	1.387	5.090
	1.831	6.580	1.487	5.210
	1.721	6.280	1.468	5.440
	1.300	5.800	1.470	5.640
	1.132	5.630	1.600	5.390
	1.119	5.330	1.480	5.430
	1.212	6.950	1.870	4.740
	1.103	6.000	1.410	5.160
	1.575	5.960	1.310	4.840
	0.894	6.560	1.290	5.730
	1.375	5.540		5.690
	1.172	6.180		5.430
	1.140			4.920
	1.250			5.260
	1.350			5.150
				5.310
				4.780
				4.890

Table 9. Raw data for the transfer of 30°C acclimated T. confusum to 18°C and then testing homogenates at 30°C

Hours after transfer	ATPase rates (ugP/mg insect/min)				Average values
6	0.593	0.497	0.428	0.464	0.497
11	0.405	0.343	0.359	0.311	0.354
19	0.610	0.544	0.442		0.532
29	0.703	0.608	0.584		0.631
36	0.434	0.438	0.379	0.390	0.410
54	0.420	0.380	0.373		0.391
60	0.568	0.543	0.448	0.491	0.510
72	0.496	0.453	0.355		0.435
84	0.631	0.472	0.481	0.444	0.532
180	0.416	0.383	0.476	0.405	0.420
216	0.347	0.450	0.484	0.466	0.437
239	0.443	0.565	0.500	0.466	0.493
312	0.647	0.545			0.496
318	0.816	0.597	0.744		0.708
378	0.431	0.525	0.486	0.502	0.486
504	0.536	0.546	0.779		0.620
600	0.572	0.698			0.633
648	0.593	0.564	0.602		0.586
746	0.669	0.529	0.697		0.632
864	0.803	0.771	0.779		0.784
885	0.371	0.328	0.399	0.408	0.376
1512	0.554	0.677	0.727		0.653

Table 10. Average values for ATPase determinations at a series of temperatures

Species	Temperature (°C)	1/T x 10^4	µg P/mg insect/min	Log_e µg P/mg insect/min
T. confusum	9.0	35.46	0.025	-3.689
30°C acclimated	18.0	34.36	0.195	-1.635
	20.5	34.07	0.158	-1.845
	24.5	33.61	0.256	-1.323
	30.0	33.00	0.697	-0.361
	35.0	32.47	0.739	-0.302
T. confusum				
18°C acclimated	10.0	35.34	0.029	-3.540
	18.0	34.36	0.274	-1.295
	22.0	33.90	0.168	-1.780
	30.0	33.00	1.058	-0.058
	35.5	32.41	0.774	-0.256
	37.5	32.20	1.250	-0.223
M. domestica	4.5	36.04	0.204	-1.590
30°C acclimated	8.5	35.53	0.498	-0.697
	11.0	35.21	1.010	+0.010
	15.0	34.72	1.570	+0.451
	18.5	34.31	1.900	+0.642
	22.0	33.90	3.490	+1.250
	25.5	33.50	3.930	+1.369
	30.0	33.00	5.730	+1.746
	38.0	32.15	9.910	+2.293
M. domestica	4.5	36.04	0.133	-2.017
15°C acclimated	9.5	35.40	0.555	-0.589
	15.0	34.72	1.330	+0.285
	20.0	34.12	2.580	+0.948
	25.0	33.56	3.600	+1.281
	30.0	33.00	5.280	+1.664
	37.5	32.23	8.830	+2.178